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MUTAGENIC RESPONSES OF SOME PETROLEUM-BASE OBSCURANTS IN THE AMES TEST

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In the Ames Test, five petroleum fractions [diesel fuel (DF) 25% distilled, DF with approximately 6% tetraethoxysilane, Jet-A, 75% bottoms product and Fog Oil, and methylene chloride extractions of combusted DF] were evaluated for their mutagenic potential. The five fractions were dissolved in ethanol containing 1% Tween 80. One hundred milligrams of the residue from combusted DF was extracted with 25 mL of methylene chloride for 24 hr. After that time, the methylene chloride was replaced with acetone by evaporation. Each of five materials was tested in the four standard tester strains; TA97, TA98, TA100 and TA102, metabolically activated (aroclor 1254 induced rat liver S9) and nonactivated. Five concentrations, ranging upwards to the approximate level of cytotoxicity, of each material were tested, and the results in each case were confirmed with a similar nonconcurrent test. Positive results were not demonstrated in any of the materials tested.

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18. Subject Terms (Continued)

Jet-A	Diesel fuel 25% distilled
75% bottoms product	Extract
Combusted diesel fuel	

PREFACE

The work described in this report was authorized under Project No.1C162622A552, Smoke and Obscurants. This work was started in October 1986 and completed in December 1987. The experimental data are recorded in laboratory notebook No. 86-0146.

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CONTENTS

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MUTAGENIC RESPONSES OF SOME PETROLEUM-BASE OBSCURANTS IN THE AMES TEST

1. INTRODUCTION

Materials used in the field as obscurants are handled by many persons including laboratory personnel, production plant employees, and military troops. One of the risks in handling these materials is the potential for producing cancer or mutations. It is of paramount importance in any toxicological evaluation to assess, as accurately as possible, the risk that the material will cause cancer or mutations in man. Risk assessment takes into account many parameters such as the probability of exposure, the frequency of exposure, the concentration of exposure, and the relative ability of the material within the system to reach the deoxyribosenucleic acid (DNA) and produce lesions.[1] The last parameter is the one with which we are concerned in this study. Fortunately, there are many in vitro and in vivo test protocols that examine various mechanisms leading to mutations and cancer. Used individually these tests are of little value. However, when a battery of carefully selected tests is used, risk assessment becomes feasible.

Among short-term tests the Ames Salmonella/Mammalian Microsome Mutagenicity Test, hereafter referred to as the Ames Test, has become a standard for detecting mutagens that may be hazardous to man.[2] Because it is rapid and economical, the Ames Test is highly desirable for screening not only relatively pure identifiable substances but also complex mixtures that may contain unidentified mutagens or carcinogens.[3]

2. BACKGROUND

2.1 The Ames Test.

The probability that an environmental mutagen will cause a mutation in species high on the evolutionary scale is low (quite low for man) because the mutagen must survive a cascade of mechanisms designed to alter, block, or remove it from the system.[1]

Bacterial and other one-cell species are ideal for testing environmental chemicals for their mutagenic potential because the extracellular portion of the cascade is not present. This greatly enhances the probability that an environmental mutagen will cause a mutation. The Ames Test, which uses several mutated strains of the bacteria, *Salmonella typhimurium*, further enhances this probability because many of the tester strains are deficient in the *uvrB* DNA repair mechanism. Some of these strains also have a deficiency in the lipopolysaccharide capsule, rendering the cell penetrable by large polycyclic hydrocarbons, and some have the pkM101 plasmid that enhances an error-prone, repair system natural to this species. Each strain is characterized by a different mutation in the histidine operon - that section of DNA that contains the genetic code leading to histidine biosynthesis. Of the four standard tester strains, TA97, TA98, TA100, and TA102, all contain the pkM101 plasmid, and all have the deficient capsule.[1,2,4,5] Tester strains TA97, TA98, and TA100 contain the *uvrB* repair deficiency; TA100 contains a base pair substitution at the *hisG46* locus and detects mutagens that cause base pair substitutions primarily at G-C pairs; TA98 contains a -1 frame-shift mutation at *hisD3052* , and TA97 contains a +1 frame-shift mutation at *hisD6610*. Both of these frame-shift mutations involve G-C pairs. Tester strain TA102 contains a base-pair substitution, that results in an ochre mutation and is the only one of the standard tester strains whose mutation in the histidine operon involves A-T pairs. This makes it useful in detecting types of mutagens such as oxidants that the other strains do not detect efficiently. Due to the mutation in the histidine operon, each of these strains requires histidine supplement for

growth. Positive mutagenic effects are seen in each strain by the reversion of its respective mutation in the histidine operon to the wild type. This allows the strain to grow in the absence of a histidine supplement.[2]

In addition, the test includes exposing the strains to the potential mutagens in the presence of S9, a 9000 x g supernatant of homogenized liver, usually from rats. Prior to being euthanized, rats are injected with aroclor 1254 intraperitoneally to induce metabolic enzymes. In the body, these enzymes are expected to metabolize foreign substances to water-soluble substances that can be excreted through the kidneys. However, in the process, carcinogens or mutagens can be formed from otherwise innocuous compounds.[2] It is the potential for this metabolic activation to mutagenicity or carcinogenicity that is evaluated on the plates containing S9.

2.2 Fog Oil (FO) Replacement.

Fog Oil, used exclusively by the U.S. Army to generate obscurant smokes, presents a logistic burden in that large quantities must be transported during military screening operations. Diesel fuel (DF) is an attractive alternative for generating smoke screens because it is in abundant supply; however, DF is more volatile than FO and can't produce as persistent a smoke. In June 1985, the Vice Chief of Staff of the U.S. Army directed that DF be fielded as a replacement for FO by October 1986. A special project team, including a representative of the U. S. Army Chemical Research, Development and Engineering Center's (CRDEC) Toxicology Divsion, was formed to expedite all actions necessary to meet this extremely ambitious schedule. Several alternative technologies were pursued, but the dissemination of combusted DF (soot) was among the most feasible. After all technologies were optimized, samples were to be provided to CRDEC's Toxicology Division for initial testing, that included rabbit eye and dermal irritation tests and Ames and Drosophila mutagenicity tests. Pending results of the initial toxicity screen and selection of the best compound/generation system, further toxicological tests including inhalation studies will be required. According to a toxicity review prepared by the Oak Ridge National Laboratory (ORNL)[6] (Oak Ridge, TN) neither FO nor DF is non-toxic. Any modifications to the DF must not make the material more toxic than the original DF or the existing FO. There is concern that the combustion to soot may increase the mutagenicity/carcinogenicity potential of the DF; therefore, emphasis is placed on this study, the backbone of most mutagenicity/carcinogenicity testing efforts.

3. MATERIALS AND METHODS

3.1 Test Materials.

3.1.1 DF, 25% Distilled.

This DF sample was the 75% bottoms product of a distillation of Philips D-2 diesel control fuel (lot G-075) and was prepared at Belvoir Fuels and Lubricants Research Facility, Southwest Research Institute (SwRI)[7] (San Antonio, TX). The fuel was diluted in ethanol containing 1% Tween 80 in a CRDEC laboratory so that an emulsion could be maintained when it was mixed into the aqueous environment essential to the Ames Test.

3.1.2 DF with Approximately 6% Tetraethoxysilane (DF-T)

Tetraethoxysilane (TES) was added to DF in an attempt to duplicate the smoke-generating properties of FO. The test sample contained 50 g of TES in 820 g of the test material and was dissolved in ethanol containinng 1% Tween 80.

3.1.3 FO.

The FO tested in this study was low viscosity, petroleum oil, MIL-F-12070 C (NATO Code F-62). The oil was distilled from Referee Grade DF (MIL-F-46162) by SwRI[7] and was diluted in ethanol containing 1% Tween 80 in preparation for the test concentrations.

3.1.4 JET A, 75% Bottoms Product (J-A 75).

This product, Jet-A (AL-15421-F), distilled to 75% by SwRI[7] was used instead of JP-8 because of its availability and great similarity to JP-8.[7] The product was dissolved in ethanol with 1% Tween 80.

3.1.5 Extract of Combusted DF-Soot (SE).

Diesel fuel was combusted and 100 mg of its nonvolatilized by-products were extracted with 25 mL of methylene chloride. Subsequently, the extractant was evaporated and replaced with 5 mL of acetone. Methylene chloride is an excellent extraction solvent for this purpose but is not compatible with the Ames system. Acetone was used as the diluent in preparing the test concentrations.

3.2 The Ames Test.

All procedures followed the revised methods for conducting the standard plate incorporation assay of the Ames test.[2] The materials were tested using the four standard tester strains (TA97, TA98, TA100, and TA102) both with and without metabolic activation. Metabolic activation plates each received 50 µg/plate of aroclor 1254 induced rat liver S9 from the Organon Teknica Corporation (Durham, NC). All vehicle controls were tested in triplicate; all plates receiving the test materials were tested in duplicate, and all positive controls were tested on single plates. 2-Aminoanthracene (2AA) (50 µg/plate) served as the positive control for all four tester strains on the metabolically activated plates. This material requires metabolic activation to induce mutagenic change on the Ames plates and is used as a control for activation only. Positive controls for the nonactivated plates were as follows: TA97, 1 µg/plate of ICR-191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; and TA102, 1 µg/plate of mitomycin C. Each positive control and the amount used are specific for their respective tester strain when not metabolically activated. All plate counts were done on the Artek Counter, model 880.

4. RESULTS

Data from the tests of all five test materials are found in Tables A-1 to A-10 in the Appendix. Several materials were tested concurrently and share common sets of controls. The second tests of DF and DF-T; (Tables A-2 and A-4), the first tests of FO and J-A 75 (Tables A-5 and A-7), and the second tests of FO and J-A 75 (Tables A-6 and A-8) were all tested as concurrent pairs.

The colony counts for the test materials DF, DF-T, FO, and J-A 75 compare favorably with their vehicle controls. However, data from the first SE test show elevated plate counts in the highest concentrations on TA97, TA98, and TA100 activated plates and somewhat elevated counts on nonactivated plates (Table A-9). Although, they exist in the second test of SE (Table A-10) high plate counts do not relate to plate concentration in every case (e.g., the first test). Certainly TA97 activated and TA98 nonactivated appear to be concentration related responses, but TA98 activated has elevated counts in the highest four concentrations with the highest concentration having the lowest count of the four. Similar results are seen in TA100 activated where the second highest plate concentration has, by far, the greatest count. All other plates compare favorably with the controls.

Microscopic examination of the plates in both SE tests revealed signs of cytotoxicity on the plates of highest concentration for both activated and nonactivated for all tester strains. Cytotoxicity at the second highest concentration was questionable. But in the case of TA98 activated in the second test, cytotoxicity was seen in the highest four concentrations. Although, the cytotoxicity could not be measured, it seemed to be proportional to concentration by subjective observation. Cytotoxicity was not seen in any of the other tests, however, there were microscopically small oily droplets on top of the agar in the highest two concentrations of the first FO test, the highest concentration of the second FO test, and the highest two concentrations of both J-A 75 tests.

5. DISCUSSION

Increases in colony counts in the Ames system are positive for mutagenesis if they relate to increases in concentration of the test material,[3, 8] if these concentration-related increases are reproducible in subsequent duplicate testing,[3, 8] and if they are at least 2-2 $^1/_2$ times higher than the vehicle controls.[2, 8, 9] In addition, in borderline cases, cytotoxicity (toxicity to the tester strains by the test material), which usually results in no growth on the plate, can result in high, low, or normal numbers of colonies, only some of which may be mutagenically induced. Cytotoxicity can be determined in such borderline cases by examining the background lawn in a microscope. Therefore, if the lawn is missing, or is sparse and noncontiguous compared to vehicle controls, colony counts would be unreliable in determining mutagenicity.

In some cases, the data from the tests on SE, Tables A-9 and A-10, seem to meet the criteria for mutagenicity (TA97 activated and TA98 nonactivated); and in other cases there are strong trends (i.e., TA98 activated, TA100 activated, and TA97 nonactivated). As we have seen, the plates of higher test material concentration and elevated colony counts also demonstrate cytotoxicity. Therefore, these colony counts are unreliable in determining mutagenicity of the test material. It is possible that we are seeing mutagenic effects at these higher concentrations of SE that are being masked by cytotoxicity caused by one or more mutagens present or by other nonmutagenic substances present in the crude extract. Cytotoxicity in the Ames Test is merely a limiting factor in interpreting results and has no relevance in the assessment of mutagenic risk to man. However, elevated colony counts that are manifestations of mutational events are very relevant. Persuant to efforts in fielding combusted DF as an obscurant its pyrolysis products should be fully characterized, and each component, evaluated for mutagenic/carcinogenic potential. Some of this information may be found in the literature.

Information published subsequent to the initiation of these tests indicate that with alterations to the standard protocol (e.g.) increasing the amount of S9 per plate as much as eight fold,[10] and designing extraction procedures that will yield products compatible with aqueous systems,[11,12] certain oil samples can give positive results in the Ames Test. Our goal here in using ethanol or acetone as vehicles was to bridge the gap between an aqueous environment, the Ames Test, and immiscible organics such as petroleum fractions and methylene chloride extractions. Our data do not reveal mutagenic activity in any of the materials tested in spite of the fact that one test material (SE) was analyzed* and shown to contain suspected carcinogens. The similarity between the oil samples yielding positive results when the standard protocol was altered [10,11,12] and our petroleum fractions, which did not yield positive results, is not clear. The apparent inconsistency in test results between the

* Martin, J. J., Research Directorate, U.S. Army Chemical Research, Development and Engineering Center, October 1986, unpublished data.

protocols is only one reason why thorough mutagenicity testing should include a battery of carefully selected assays, and does not rely soley on one assay. Relative to the interest in promoting one or more of these substances as a viable smoke candidate, additional mutagenicity testing is indicated. Initially, other short-term in vitro tests should be considered and followed by more advanced testing on rodents if the data warrant and interest continues.

6. CONCLUSIONS

Using the Ames standard plate incorporation assay, mutagenicity could not be demonstrated in any of the five test materials. Combusted DF was shown to have chemical classes that contain strong mutagens. Although elevated plate counts were seen at the high concentrations in some tester strains, cytotoxicity was also seen, obscuring mutagenic effects that may have been present. Other short-term in vitro tests followed by the more advanced rodent tests are indicated for any of the five materials being further considered as FO replacements.

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APPENDIX
TEST DATA

Table A-1. Petri Plate Colony Counts in an Ames Test of Diesel Fuel That Was 25% Distilled[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	144 ± 35.5[d]	44 ± 4.2	171 ± 20.5	296 ± 75.5
15.0 µL/plate	170 ± 0.7	44 ± 0.0	147 ± 5.2	298 ± 40.3
1.5 µL/plate	188 ± 3.5	55 ± 6.4	157 ± 8.5	337 ± 56.7
0.15 µL/plate	170 ± 30.4	56 ± 5.0	166 ± 12.8	320 ± 41.7
.015 µL/plate	158 ± 39.5	48 ± 6.4	144 ± 1.4	290 ± 88.4
.0015 µL/plate	161 ± 6.4	46 ± 1.4	155 ± 17.0	262 ± 13.4
Positive Controls[e]	952	153	1612	832
NONACTIVATED				
Vehicle Control	163 ± 11.0	44 ± 4.0	164 ± 9.9	233 ± 50.0
15.0 µL/plate	186 ± 1.4	46 ± 2.1	156 ± 21.2	242 ± 44.5
1.5 µL/plate	167 ± 14.8	44 ± 4.2	183 ± 22.6	218 ± 12.7
.15 µL/plate	164 ± 2.8	39 ± 5.7	134 ± 60.8	211 ± 10.6
.015 µL/plate	176 ± 2.1	34 ± 5.7	131 ± 15.6	216 ± 26.2
.0015 µL/plate	167 ± 5.7	29 ± 5.7	192 ± 1.4	234 ± 1.4
Positive Controls	566	308	980	643

a This is the first of two test runs.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Ethanol containing 1% Tween 80 was used as the vehicle to maintain an emulsion.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.

Table A-2. Petri Plate Colony Counts in an Ames Test of Diesel Fuel That Was 25% Distilled[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	203 ± 13.6[d]	31 ± 8.7	193 ± 8.2	293 ± 26.4
15.0 µL/plate	184 ± 12.7	38 ± 4.2	178 ± 48.8	250 ± 31.1
1.5 µL/plate	200 ± 16.3	40 ± 2.1	196 ± 6.4	317 ± 21.9
0.15 µL/plate	184 ± 9.2	42 ± 4.9	186 ± 3.5	295 ± 2.1
.015 µL/plate	173 ± 4.2	32 ± 14.1	203 ± 18.4	308 ± 28.3
.0015 µL/plate	194 ± 2.1	33 ± 6.4	195 ± 7.1	298 ± 87.0
Positive Controls[e]	1238	1472	1231	604
NONACTIVATED				
Vehicle Controls	199 ± 41.9	32 ± 5.5	269 ± 23.3	304 ± 15.5
15.0 µL/plate	212 ± 30.4	40 ± 2.8	201 ± 16.3	257 ± 46.0
1.5 µL/plate	232 ± 12.0	25 ± 2.8	287 ± 4.2	281 ± 25.5
0.15 µL/plate	200 ± 19.8	21 ± 4.2	214 ± 5.7	263 ± 26.9
.015 µL/plate	207 ± 6.4	42 ± 7.8	269 ± 0.7	277 ± 20.5
.0015 µL/plate	225 ± 26.5	25 ± 1.4	312 ± 21.2	326 ± 9.9
Positive Controls	503	346	661	596

a This is the second of two test runs and confirms the results of the first.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Ethanol containing 1% Tween 80 was used as the vehicle to maintain an emulsion.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.

Table A-3. Petri Plate Colony Counts in an Ames Test of Diesel Fuel Containing the Additive Tetraethoxysilane[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	142 ± 18.0[d]	61 ± 9.8	156 ± 6.8	358 ± 19.7
15.0 µL/plate	146 ± 7.1	52 ± 7.1	125 ± 6.4	356 ± 12.7
1.5 µL/plate	147 ± 21.9	59 ± 4.9	153 ± 2.8	352 ± 12.7
0.15 µL/plate	157 ± 21.2	58 ± 0.7	150 ± 21.9	379 ± 4.9
.015 µL/plate	147 ± 1.4	56 ± 5.7	147 ± 7.1	344 ± 63.6
.0015 µL/plate	165 ± 0.0	67 ± 13.4	163 ± 13.4	351 ± 20.5
Positive Controls[e]	1714	968	1298	914
NONACTIVATED				
Vehicle Control	142 ± 12.8	59 ± 4.3	164 ± 8.1	362 ± 1.0
15.0 µL/plate	131 ± 14.1	79 ± 6.4	127 ± 7.1	356 ± 9.9
1.5 µL/plate	155 ± 19.1	57 ± 9.9	180 ± 8.5	343 ± 20.5
0.15 µL/plate	164 ± 11.3	65 ± 7.0	193 ± 6.4	339 ± 34.6
.015 µL/plate	174 ± 24.0	64 ± 22.6	173 ± 7.8	341 ± 25.5
.0015 µL/plate	168 ± 16.3	70 ± 26.9	154 ± 6.4	325 ± 33.2
Positive Controls	541	151	572	929

a This is the first of two test runs. The diesel fuel contained approximately six percent tetraethoxysilane by weight.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Ethanol containing 1% Tween 80 was used as the vehicle to maintain an emulsion.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.

Table A-4. Petri Plate Colony Counts in an Ames Test of Diesel Fuel Containing the Additive Tetraethoxysilane[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	200 ± 15.6[d]	31 ± 8.7	193 ± 8.2	290 ± 26.0
15.0 µL/plate	95 ± 9.9	41 ± 4.8	148 ± 21.2	296 ± 18.4
1.5 µL/plate	157 ± 2.1	49 ± 1.4	172 ± 5.7	325 ± 2.8
0.15 µL/plate	187 ± 0.7	49 ± 4.5	184 ± 45.3	350 ± 38.2
.015 µL/plate	179 ± 22.6	50 ± 6.4	222 ± 9.9	341 ± 18.4
.0015 µL/plate	205 ± 20.5	50 ± 4.2	227 ± 5.7	348 ± 7.1
Positive Controls[e]	230	1472	1231	604
NONACTIVATED				
Vehicle Control	199 ± 41 9	31 ± 5.5	269 ± 23.3	304 ± 15.5
15.0 µL/plate	146 ± 36.1	49 ± 14.8	163 ± 31.8	285 ± 2.8
1.5 µL/plate	184 ± 3.5	48 ± 3.5	222 ± 33.2	318 ± 19.8
0.15 µL/plate	209 ± 14.8	47 ± 5.7	248 ± 12.7	319 ± 26.9
015 µL/plate	194 ± 23.3	37 ± 2.1	326 ± 4.9	319 ± 15.6
.0015 µL/plate	218 ± 24.0	44 ± 3.5	279 ± 27.6	316 ± 8.5
Positive Controls	583	346	661	596

a This is the second of two test runs and confirms the results of the first. The diesel fuel contained approximately six percent tetraethoxysilane by weight.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Ethanol containing 1% Tween 80 was used as the vehicle to maintain an emulsion.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.

Table A-5. Petri Plate Colony Counts in an Ames Test of Fog Oil[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	252 ± 30.3[d]	53 ± 11.9	161 ± 18.2	159 ± 49.0
15.0 µL/plate	311 ± 18.4	64 ± 8.5	239 ± 16.4	276 ± 40.3
1.5 µL/plate	278 ± 17.0	52 ± 5.7	202 ± 3.5	278 ± 6.4
0.15 µL/plate	254 ± 39.6	54 ± 1.4	198 ± 19.8	236 ± 53.0
.015 µL/plate	270 ± 1.4	60 ± 3.5	172 ± 4.2	198 ± 35.4
.0015 µL/plate	255 ± 68.6	61 ± 2.8	187 ± 9.2	241 ± 61.2
Positive Controls[e]	1093	1118	1091	796
NONACTIVATED				
Vehicle Control	203 ± 19.1	42 ± 7.4	173 ± 17.1	267 ± 22.3
15.0 µL/plate	327 ± 28.3	50 ± 4.2	216 ± 18.4	227 ± 9.9
1.5 µL/plate	278 ± 28.3	53 ± 0.0	203 ± 33.9	228 ± 33.9
0.15 µL/plate	253 ± 9.9	64 ± 0.0	215 ± 12.0	212 ± 31.1
.015 µL/plate	246 ± 4.2	53 ± 10.6	209 ± 12.0	188 ± 28.3
.0015 µL/plate	276 ± 6.4	55 ± 8.5	210 ± 9.9	185 ± 19.1
Positive Controls	620	335	822	1025

a This is the first of two test runs.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Ethanol containing 1% Tween 80 was used as the vehicle to maintain an emulsion.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.

Table A-6. Petri Plate Colony Counts in an Ames Test of Fog Oil[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	347 ± 23.6[d]	52 ± 8.1	159 ± 10.1	321 ± 15.5
15.0 µL/plate	436 ± 79.9	74 ± 19.1	278 ± 12.7	356 ± 46.0
1.5 µL/plate	432 ± 36.1	67 ± 5.7	262 ± 0.0	370 ± 1.0
0.15 µL/plate	387 ± 27.6	56 ± 1.4	206 ± 0.0	344 ± 8.5
.015 µL/plate	53 ± 11.3	50 ± 11.3	183 ± 7.8	358 ± 1.6
.0015 µL/plate	392 ± 7.0	48 ± 7.1	167 ± 12.0	317 ± 46.7
Positive Controls[e]	907	1485	1348	753
NONACTIVATED				
Vehicle Control	322 ± 40.6	46 ± 10.6	172 ± 8.1	340 ± 14.8
15.0 µL/plate	399 ± 20.5	77 ± 15.6	226 ± 7.1	260 ± 7.1
1.5 µL/plate	390 ± 24.7	60 ± 9.9	183 ± 6.4	296 ± 4.9
0.15 µL/plate	429 ± 5.7	51 ± 14.8	181 ± 19.1	275 ± 0.7
015 µL/plate	414 ± 43.8	55 ± 1.4	187 ± 10.6	284 ± 37.5
.0015 µL/plate	404 ± 16.3	51 ± 3.5	193 ± 0.7	300 ± 2.1
Positive Controls	810	377	975	1079

a This is the second of two test runs and confirms the results of the first.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Ethanol containing 1% Tween 80 was used as the vehicle to maintain an emulsion.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102; 1 µg/plate of mitamycin C.

Table A-7. Petri Plate Colony Counts in an Ames Test of Jet A, 75% Bottoms Product[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	225 ± 21.5[d]	53 ± 11.9	159 ± 19.7	159 ± 49.0
15.0 µL/plate	189 ± 38.9	47 ± 4.2	114 ± 14.1	266 ± 24.7
1.5 µL/plate	206 ± 66.5	51 ± 1.4	134 ± 14.8	269 ± 63.6
0.15 µL/plate	218 ± 33.9	50 ± 9.2	164 ± 25.2	261 ± 34.6
.015 µL/plate	230 ± 21.2	53 ± 2.1	145 ± 7.1	249 ± 34.6
.0015 µL/plate	221 ± 38 2	52 ± 7.1	167 ± 12.7	233 ± 30.4
Positive Controls[e]	1093	1118	1091	796
NONACTIVATED				
Vehicle Control	204 ± 18.6	42 ± 7.4	173 ± 17.1	267 ± 22.3
15.0 µL/plate	154 ± 24.7	46 ± 7.1	88 ± 9.9	252 ± 7.8
1.5 µL/plate	164 ± 26.2	58 ± 23.3	99 ± 5.7	232 ± 9.9
0.15 µL/plate	173 ± 42.4	45 ± 2.8	127 ± 24.0	230 ± 22.6
.015 µL/plate	202 ± 33.9	50 ± 4.2	141 ± 21.9	257 ± 12.0
.0015 µL/plate	201 ± 29.7	48 ± 5.7	158 ± 5.7	239 ± 37.5
Positive Controls	620	335	822	1025

a This is the first of two test runs.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Ethanol containing 1% Tween 80 was used as the vehicle to maintain an emulsion.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.

Table A-8. Petri Plate Colony Counts in an Ames Test of Jet A, 75% Bottoms Product[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	347 ± 23.6[d]	54 ± 5.2	159 ± 10.1	321 ± 15.5
15.0 µL/plate	257 ± 56.6	48 ± 11.3	120 ± 3.5	354 ± 34.6
1.5 µL/plate	303 ± 56.6	49 ± 0.7	153 ± 15.6	356 ± 50.9
0.15 µL/plate	311 ± 59.4	50 ± 2.8	146 ± 27.6	333 ± 41.0
.015 µL/plate	339 ± 1.4	51 ± 6.4	153 ± 22.6	333 ± 41.0
.0015 µL/plate	318 ± 31.0	55 ± 13.4	156 ± 4.9	349 ± 32.5
Positive Controls[e]	907	1485	1348	753
NONACTIVATED				
Vehicle Control	322 ± 5.7	46 ± 10.6	172 ± 8.1	340 ± 14.8
15.0 µL/plate	133 ± 5.7	45 ± 6.4	118 ± 12.0	284 ± 5.7
1.5 µL/plate	161 ± 38.2	52 ± 2.8	147 ± 12.7	311 ± 13.4
0.15 µL/plate	236 ± 26.2	46 ± 2.1	155 ± 19.1	306 ± 6.4
.015 µL/plate	304 ± 18.4	53 ± 9.9	182 ± 14.1	193 ± 12.0
.0015 µL/plate	334 ± 70.7	44 ± 6.4	181 ± 26.9	295 ± 12.7
Positive Controls	810	377	975	1079

a This is the second of two test runs and confirms the results of the first.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Ethanol containing 1% Tween 80 was used as the vehicle to maintain an emulsion.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.

Table A-9. Petri Plate Colony Counts in an Ames Test of an Extract of Combusted Diesel Fuel[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	129 ± 6.1[d]	26 ± 4.0	112 ± 16.9	290 ± 7.0
Stock Solution	388 ± 18.4	106 ± 23.3	287 ± 16.9	399 ± 29.7
SS x 10^{-1}	268 ± 23.3	38 ± 0.0	245 ± 5.7	312 ± 43.8
SS x 10^{-2}	165 ± 22.6	36 ± 2.8	179 ± 11.3	295 ± 32.5
SS x 10^{-3}	150 ± 7.8	26 ± 3.5	152 ± 24.7	283 ± 29.0
SS x 10^{-4}	148 ± 17.7	33 ± 7.8	128 ± 5.7	258 ± 27.6
Positive Controls[e]	907	1485	1348	753
NONACTIVATED				
Vehicle Control	120 ± 31.2	33 ± 12.9	143 ± 17.2	302 ± 15.5
Stock Solution	251 ± 31.1	101 ± 8.5	244 ± 14.8	367 ± 12.7
SS x 10^{-1}	168 ± 22.6	40 ± 8.5	182 ± 42.8	358 ± 5.7
SS x 10^{-2}	125 ± 1.4	27 ± 10.6	161 ± 12.0	321 ± 2.8
SS x 10^{-3}	110 ± 8.5	34 ± 2.1	151 ± 18.4	286 ± 14.1
SS x 10^{-4}	147 ± 26.9	27 ± 5.7	152 ± 9.9	310 ± 9.2
Positive Controls	1604	1546	1934	533

a This is the first of two test runs. The combusted diesel fuel was extracted with methylene chloride which was replaced with 5mL of acetone.
b Metabolically activated with aroclor 1254 induced rat liver S9.
c Acetone was used as the vehicle control.
d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.
e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.

Table A-10. Petri Plate Colony Counts in an Ames Test of an Extract of Combusted Diesel Fuel[a]

ACTIVATED[b]	TA97	TA98	TA100	TA102
Vehicle Control[c]	252 ± 59.5[d]	41 ± 8.5	207 ± 48.7	334 ± 41.8
Stock Solution	772 ± 112.4	93 ± 64.3	296 ± 32.5	312 ± 20.5
5SS x 10^{-1}	352 ± 212.1	136 ± 32.5	487 ± 103.2	407 ± 36.8
SS x 10^{-1}	176 ± 12.7	115 ± 18.5	359 one plate	349 ± 12.7
5SS x 10^{-2}	218 ± 59.4	100 ± 25.5	216 ± 75.7	327 ± 75.7
SS x 10^{-2}	177 ± 26.9	44 ± 2.1	282 ± 60.1	305 ± 88.4
Positive Controls[e]	1179	1848	1149	892
NONACTIVATED				
Vehicle Control	189 ± 46.3	29 ± 11.2	181 ± 18.1	332 ± 19.4
Stock Solution	303 ± 8.5	75 ± 0.7	203 ± 34.6	357 ± 8.5
5SS x 10^{-1}	190 ± 21.9	56 ± 4.9	236 ± 24.0	331 ± 42.4
SS x 10^{-1}	192 ± 5.7	29 ± 5.7	209 ± 172.5	342 ± 53.0
5SS x 10^{-2}	211 ± 31.3	28 ± 9.2	173 ± 7.8	360 ± 24.0
SS x 10^{-2}	193 ± 48.8	29 ± 2.1	215 ± 14.1	278 ± 26.9
Positive Controls	527	426	879	607

a This is the second of two test runs and confirms the results of the first. The combusted diesel fuel was extracted with methylene chloride which was replaced with 5mL of acetone.

b Metabolically activated with aroclor 1254 induced rat liver S9.

c Acetone was used as the vehicle control.

d The data are expressed as means of triplicate plates for the vehicle controls, and means of duplicate plates for the test material, ± S.D.

e Positive controls (single plates): 50 µg/plate of 2-aminoanthracene for all activated plates. Nonactivated plates: TA97, 1 µg/plate of ICR 191; TA98, 1 µg/plate of 2-nitrofluorene; TA100, 1 µg/plate of sodium azide; TA102, 1 µg/plate of mitamycin C.